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# Potato Production Impacts of the Association Trichodorids and TRV in Naturally Infected Fields

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**Abstract:** An investigation was conducted to determine the effect of soil-born *Tobacco rattle virus* (TRV) isolates on yield and some quality attributes of 8 potato cultivars (Agata, Amorosa, Almera, Cunéria, Kondor, Picasso, Raja and Stemster) in Portuguese growing areas. Preliminary results revealed that some attributes (canopy growth, total yield, tuber numbers and yield within size grades, tuber spraing symptoms and secondary growths) are severely affected in the sites with viruliferous trichodorids transmitting TRV. It was shown that TRV has real conditions for survival and cause economic impact in potato crop in these regions. These results have potential interest to all potato growing countries.

*Keywords:* potato cultivars, tuber symptoms, *Tobacco rattle virus*, tobravirus, transmission, trichodorids, vector nematodes

## 1. INTRODUCTION

The potato is the world's major root and tuber crop in temperate, sub-temperate and tropical regions under different socio-economic environments and is consumed almost daily by more than a billion people (FAO, 2009). One of the threats to potato productivity is the damage caused by more than 35 different viruses (Loebenstein *et al.*, 2001). *Tobacco rattle virus* (TRV) is among the viruses considered the most economically important in most susceptible cultivars worldwide (Brown, 1997; Salazar, 2003; Taylor and Brown 1997). Although having a restricted distribution it can cause significant yield losses where it occurs or pose significant risks if introduced to other regions.

In susceptible cultivars, TRV induces stem mottle, tuber distortions and tuber flesh disfiguration, named 'spraing' or 'corky ringspot' (CRS), usually consisting of brown necrotic arcs and spots. It is also responsible for the yield reduction (Robinson and Harrison, 1989). In UK, the control of potato spraing disease is responsible for the main consumption of agrochemicals in potato production areas. Spraing incidence in only 1 to 2% of the lots can lead to the rejection of all crop by supermarkets and potato processors. In North America and Europe, there has been a significant increase on the concern and attention dedicated to this phytosanitary problem (MacFarlane *et al.,* 2002), particularly in seed potato production areas, where TRV is the major soil-born disease (Weingartner, 2001).

TRV has a wide host range, is naturally transmitted by soil living ectoparasitic trichodorid nematodes (*Nanidorus*, *Paratrichodorus* and *Trichodorus* species) and occurs as a vast series of serologically distinguishable strains. Most TRV strains are transmitted by a particular trichodorid species (Brown and Weischer, 1998). The virus and its associated vector nematodes occur world-wide, being particularly prevalent in Europe and North America.

In Portugal, TRV was reported for the first time by Ploeg and Brown (1997). Later, a field survey for the association of TRV-trichodorids in potato fields was carried out in the Northwest of the country (Duarte *et al.*, 2001). The sampling, based on the results of an inquiry for TRV-like symptomatology in potato tuber flesh, revealed the trichodorids prevalence in 75% of the sites. TRV isolates were recovered from viruliferus trichodorids using virus transmission tests (Brown *et al.*, 1989; Brown, 1997) from 5% of the sampled sites. Vector species are disseminated in the sampled regions, which have real conditions for TRV survival (not yet published).

The reduction in the marketability of the harvested tubers, due to quality and quantity deterioration caused by viruses has been referred by several authors, but precise data regarding the crop production economical losses due to tobravirus diseases are not available.

The aim of the present research is to determine the effect of soil-born TRV isolates on yield and some quality attributes of potato tubers cultivars in potato growing areas in northern and central Portugal. We here report for the first time some preliminary results from this study.

## 2. MATERIALS AND METHODS

Field trials were performed in two sites where soil patches with naturally viruliferous trichodorids had been previously identified. The TRV-NmC9-2 and TRV-PdC36-2 isolates of unknown serotypes are present at sites C-9 and C-36, respectively, which maintain mixed populations of trichodorid species (Table 1). The typical crop rotation adopted includes potato, been or maize during Spring/ Summer, and resting field, fodder or cabbage during Autumn/ Winter.

Eight potato cultivars (Agata, Amorosa, Almera, Cunéria, Kondor, Picasso, Raja, Stemster) were selected for this experiment due to their present or potential representation on Portuguese market, different degrees of maturity and TRV resistance. Kondor and Raja are cultivars with high and very high resistance to TRV, respectively (ECPD, 2001).

**Table 1. Characterization of the experimental sites**

| | Site code nº | |
|---|---|---|
| | **C-09** | **C-36** |
| **Location** | **Vila Verde**, Oliveira do Bairro, Aveiro | **Carromeu,** Mira, Coimbra |
| **TRV isolates** | TRV-NmC9-2 | TRV-PdC36-2 |
| **Trichodorid species** | *P. divergens* + *P. hispanus*+ *N. minor* + *N. nanus* | *P. anemones* + *P. divergens* + *N. minor* + *N. nanus* |
| **Altitude (m)** | 40 | 40 |
| **Soil use** | Arbustive | Tilled |
| **Precipitation (mm)** | 1000 - 1200 | 800 - 1000 |
| **Average daily temperature (ºC)** | 15 - 17.5 | 12.5 - 14.9 |
| **Evapotranspiration (mm)** | 600 - 700 | 600 - 700 |
| **Watershed** | Vouga | Mondego |
| **Texture class** | Sandy | Sandy |
| **Soil structure** | Granulose (e) | Granulose (e) |
| **Organic matter (%)** | 1,5 | 1,3 |
| **Soil pH (H2O)** | 5,4 | 5,8 |
| **$P_2O_5$ (mg/ 1000g)** | 281 | 299 |
| **$K_2O$ (mg/ 1000g)** | 84 | 97 |

A randomised block design, with a layout of four blocks with eight treatments each, was set up in soil patches with viruliferous trichodorids to ensure the natural TRV infection of potato cultivars seeded. Each cultivar was planted in a plot spaced by 50 and 40cm in-between and within the rows, respectively. Guard rows were kept at the edges of the trial. Healthy certified seed potatoes (supplied by Eurobatata) were sprouted in wood boxes. The sprouts had about 1 cm long when the entire tubers were hand planted in 30 cm width ridges, in a planting spacing of 40 cm between rows and 35 cm within the row.

Soil was hand tilled, without manure incorporation. Fertilization had into account both the result of soil analysis and the reference value of potato export of 25000 kg of tubers/ ha that corresponds to 103.47 and 210 kg/ha of N, $P_2O_5$ and $K_2O$, respectively (Santos, 1976). Furrow irrigation was used when necessary, including the tuberisation period. Weeds were hand controlled. Several sprayings against late blight (*Phytophthora infestans* (Mont.) de Barry with mancozebe+metalaxil (160+20g of ai/hl) and the Colorado beetle (*Leptinotarsa decenlineata* Say) with clorfenvinfos (24g of ai/ hl), beta-ciflutrina (1.25g of ai/ hl) and imidaclopride (1.25g of ai/hl) were applied using a lever-operated knapsack sprayer, debiting 800 l/ha and producing a course spray, with an average droplet diameter over 400 µm. Crop development was assessed by 'plant maturation', scored from 1 to 7 (1, very immature; 7, very mature) and by 'canopy development', scored from 1 to 7 (1, very small; 7, very large).

After harvesting, daughter tubers were assessed for: total yield, tuber numbers and tuber yield within size grades (< 35mm, 35-45mm, 45-65mm, >65mm) and 'tuber appearance' *i.e.* secondary growths, TRV like-symptoms in the skin and flesh, within size grades. Two aspects were recorded: i) the number of daughter tubers per plot bearing spraing symptoms, regardless its severity; ii) the severity of symptoms of each individual sliced tuber, scored from 1 to 9 (1, severe TRV-like symptoms; 2 to 4, less severe TRV-like symptoms; 5 and 6, necrosis of doubtful origin; 7 and 8, brown spots not typical of TRV-like symptomatology; 9, symptomless). Data were converted into yield per hectare and treated to estimate the following attributes used by Robinson *et al*. (2004):

The **incidence of spraing symptoms** (ISS), as the percentage of tubers bearing symptoms, regardless its severity, relative to the total number of tubers in each plot.

The **relative incidence of spraing symptoms** (rISS), as the proportion of symptomatological incidence of each cultivar (per plot) relative to the greatest incidence for any cultivar at that site, multiplied by 100.

The **severity of spraing symptoms per plot** (SSSp), as the mean of individual tuber scores, within the three units of the lowest score in of each plot.

The **severity of spraing symptoms per cultivar** (SSSc). Calculated from the plot scores, using the same criteria as described for plots.

The **severity of spraing symptoms of X% of the worse tubers** ($SSSx\%_c$). Calculated from the mean scores within X% of the lowest scores of tubers of each cultivar.

The **combined score**. Calculated for each cultivar at each site.

F and t statistic tests were applied to the data.

## 3. RESULTS AND DISCUSSION

The assessment of plant development following emergence revealed overwhelming evidences of real differences in canopy exuberance among cultivars within each trail (Fig. 1). The plants showed either high dependence apparently on those particular edaphoclimatic conditions or on the TRV isolates present in soil, or both.

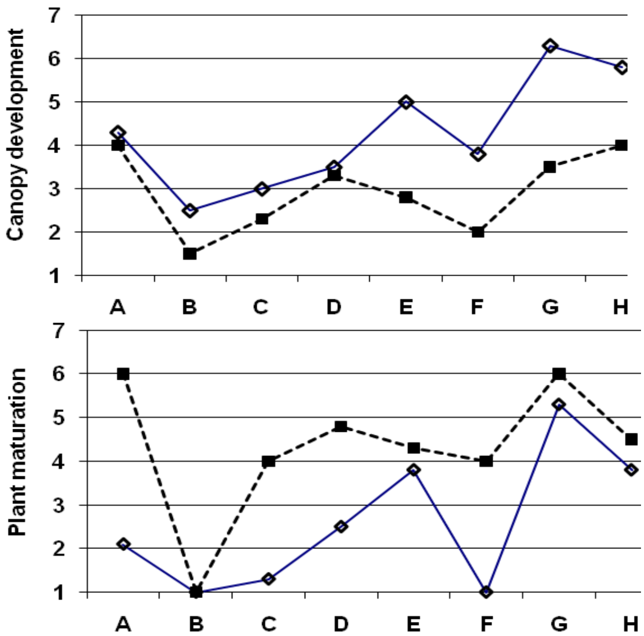


**Fig. 1.** Canopy development (upper) and plant maturation (lower) of potato cultivars in sites in sites C-9 (—◇—) and C-36 (--■--).

Code for cultivars: **A**, Kondor; **B**, Almera; **C**, Amorosa; **D**, Raja; **E**, Agata; **F**, Cunera; **G**, Stemster, **H**, Picasso.

Cultivar B developed a severe symptomatology consisting of generalized mottle in leaves, on all stems, together with dwarfing to the plant, and later would reveal to be the less productive cultivar. It showed the lower canopy development and the poorest maturation, regardless of the site of the experiment. Conversely, cultivars G and H presented the highest average values.

The canopy development of all cultivars within site C-9 is positively related with total yield (Fig. 2), being significantly higher than in site C-36. Conversely, in site C-36 there is no correlation between these factors, which indicates the determinate influence of another factor on yield, possibly the virus isolate and or site effect.

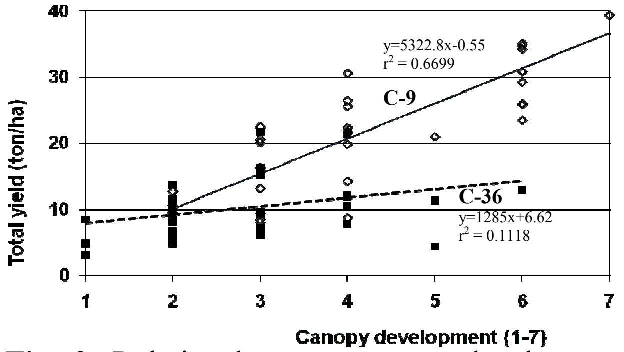


**Fig. 2.** Relation between canopy development and yield in sites C-9 (—◇—) and C-36 (--■--).

At site C-9 the differences among cultivars on both tuber yield and weight proportion of lower size graded tubers (Fig. 3) had significant statistical support. The total yield comparison amongst the eight cultivars revealed overwhelming evidences of real differences among them (0.1% significance point of the F (10.8) for 7 and 21 degrees of freedom). T-tests, revealed three groups of cultivars, without significant differences within each group, being in increasing order of productivity: i) B, C, and D; ii) A, F; iii) E, G and H, with an average yield of 13000 kg/ha, 22000 kg/ha, and 31200 kg/ha, respectively. The increases between the three referred groups of 9050 and 9180 kg/ha respectively, have standard errors of 2394.4, based on 21 df. The evidence obtained indicates that TRV isolate PmC9-2 is able to become pathogenic in all tested cultivars, including A and D considered resistant (Fig. 4). The main putative effect of this isolate on cultivars B, C and D, with low spraing incidence, was a reduced yield. Conversely, cultivars A, G and H that produced a higher proportion of spraing tubers (25.4% compared with 7.0% in the remaining cultivars) had higher yield. Moreover, these three cultivars produced the heaviest average potatoes (30/51/46 vs 75/64/68 g/tuber, respectively). Cultivar F, yielded medially and produced the less affected tubers, while cultivar E produced low average tuber weight (51 g/tuber), but high yield. No TRV-like symptoms were found on the flesh of distorted tubers.

At site C-36 four cultivars remained spraing free (A, B, C and H), apparently resisting to TRV-PdC36-2 isolate. Cultivar D was the most productive, but with a higher proportion of low graded tubers (27%) coupled with a reduced average tuber weight (34 g/tuber). It developed the higher proportion of affected tubers (11.5%), yielding the higher proportion of distortions (7.5%), plus 4% of spraing tubers. Cultivars B, F and G had the lower yields, a very high proportion of lower graded potatoes (34/44/35%) and reduced average tuber weight (34/31/31 g/tuber), respectively. Cultivar E produced the highest proportion of low graded tubers (56%). Cultivars C and E also developed similar percentage of deformed tubers; although, tubers of cultivar C were spraing-free. Cultivar F had the highest spraing incidence (6.8%).

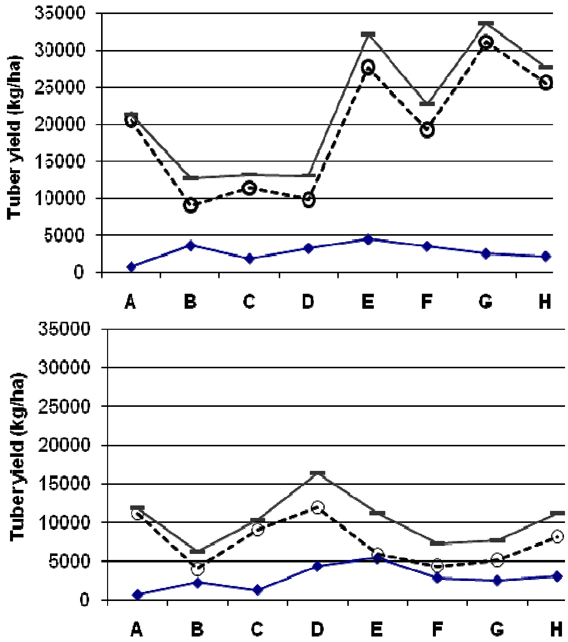


**Fig. 3.** Yield components (size grade < 35 mm, —◆— ; size grade > 35 mm, --⊖-- , total production, ——) of potato cultivars at site C-9 (upper) and C-36 (lower). For cultivars code *cf.* legend of Fig. 1.

No cultivar remained spraing-free in both sites. TRV-like symptomatology in tuber flesh varied from the typical spraing and CRS, to brown spots, dark centre and pinpoint lesions. This phenotype was statistically significantly higher in site C-9 than in C-36, where all tested cultivars developed these symptoms, although with a distinct incidence (Fig. 4). These results might indicate that isolate TRV-PmC9-2 is the most virulent in terms of spraing symptomatology. Conversely, no differences were observed between sites regarding the secondary growths of tubers (except for cultivar G). Cultivar B did not develop distorted tubers. Conversely, cultivars D and G and C, D and E were significantly most affected with this phenotype than the remaining cultivars in sites C-9 and C-36, respectively. Cultivar D was the most affected with this defect in site C-9.

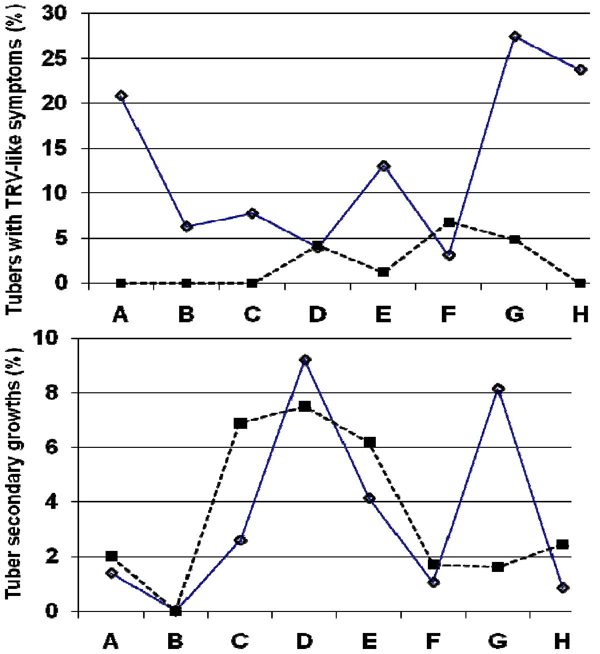


**Fig. 4.** Incidence of tubers sparing symptoms (upper) and secondary growths (lower) in sites C-9 (—◆—) and C-36 (--■--). For cultivars code *cf.* legend of Fig. 1.

The SSSc ranking of the cultivars (Table 2) is different in the two sites, indicating a different response of the plant genome to different conditions, including the TRV isolate. Similarly, the combined score ranked the cultivars differently in both sites. For example, cultivar H which was severely affected at site C-9 remained symptomless at site C-36, while cultivar H was ranked in opposition.

Cultivar ***Kondor*** (A), which according to ECPD (2001) is highly resistant to TRV, was ranked in a medium productive group. It yielded a small proportion of lower size graded tubers (3/6% in site C-9/C-36) respectively; a high proportion of tubers with size grade 45-65 mm diameter (73/55%) and a high average tuber weight (75/61 g/tuber). A low proportion of tubers were affected with secondary growths (1.4/2.5%) and a higher one with spraing (21/0%). These results show some evidence that *Kondor* is not resistant to all TRV isolates, namely to isolate present in site C-9, as it appears to be quite vulnerable to produce tuber flesh corky symptoms induced by TRV-PmC9-2 isolate.

**Table 2.** Ranking of eight potato cultivars per site, based on a combined score for TRV-like symptomatology in tuber flesh.

| Site | Cultivar | Incidence (%) | Assessment of severity of TRV-like symptomatology in tuber flesh | |
|---|---|---|---|---|
| | | | Sev. (**SSSc**) | Comb. |
| **C-9** | **D** | *2.5* | 4.0 | **9** |
| | **F** | *2.6* | 4.0 | **9** |
| | **B** | *5.6* | 3.1 | **8** |
| | **C** | *13.6* | 4.0 | **7** |
| | **E** | *10.7* | 1.9 | **6** |
| | **A** | *21.1* | 2.2 | **4** |
| | **G** | *29.7* | 3.7 | **4** |
| | **H** | *27.7* | 1.5 | **2** |
| **C-36** | **A** | *0.0* | 9.0 | **9** |
| | **B** | *0.0* | 9.0 | **9** |
| | **C** | *0.0* | 9.0 | **9** |
| | **H** | *0.0* | 9.0 | **9** |
| | **E** | *1.6* | 4.0 | **8** |
| | **D** | *3.9* | 5.0 | **7** |
| | **G** | *4.8* | 5.0 | **6** |
| | **F** | *6.6* | 4.0 | **4** |

**Sev.**, SSSc, scored 1-9; *Incid.*, rISS (percentage of symptomatic tubers in the total of yield tubers); **Comb.**, Combined score (scored 1-9).

Cultivar ***Almera*** (B) was the less productive cultivar (9457 kg/ha), both in total and marketable valuable fraction. It yielded 19% of lower size graded tubers (34%), low proportion of tubers with size grade 45-65 mm diameter (31/14%) and the lightest average tuber (30/34 g/tuber). Apparently, the TRV infection caused a great effect on emergence and canopy development. Similarly to what Dale *et al.* (2000) suggested for cultivar Wilja, it appears that *Almera* is susceptible to these isolates that affect the efficiency of interception of solar radiation, the conversion into dry weight, the subsequent accumulation of tubers dry weight and, consequently, the reduction of final yield. The high proportion of lower size graded tubers (reduced tuber size, coupled with a tuber increased number), suggests the production difficulties of TRV infected plants. However, this cultivar is one of the less affected by both secondary growths (0%) and TRV-like symptoms in tuber flesh (6/0%). These results indicate that *Almera* is not resistance to these TRV isolates.

Cultivar ***Amorosa*** (C) is ranked in the less productive group. It yielded a low proportion of lower size graded tubers (15/12%) and a proportion of tubers with size grade 45-65 mm diameter of 58/18%. It was the most affected with both secondary growths (5/8%) and TRV-like symptoms in tuber flesh (14/0%). These evidences show that among the tested cultivars, *Amorosa* appeared to be quite vulnerable to produce tuber flesh corky symptoms and distortions due to TRV-PmC9-2.

Cultivar **Raja** (D), which according to ECPD (2001) is also highly TRV resistant was here included in the medially productive group of cultivars, with a high proportion of lower size graded tubers produced (27/27%) and low average tuber weight (46/34 g/tuber). It developed the highest proportion of secondary growths in tubers (10/8%), but in contrast, a low percentage of spraing symptoms (3/4%). These results show some evidences that cultivar Raja is not resistance to these TRV isolates.

Cultivar ***Agata*** (E) was ranked in the most productive group (21692 kg/ha), although in site C-9 yield was nearly threefold that of? site C-36. The proportion of lower size graded tubers produced was much lower in site C-9 (14%) than in site C-36 (56%). *Agata* was the tested cultivar with the highest proportion of these small tubers, belonging simultaneously to the group with the lightest tubers (average of 55 g/tuber). It produced 5/7% of tubers with secondary growths. TRV-like symptomatology had an incidence of 11/2%. *Agata* is not resistant to these TRV isolates: TRV-PmC9-2; induced tuber distortion and CRS symptoms and TRV-PdC36-2 caused a severe increase of lower graded tubers.

Cultivar ***Cunera*** (F) was ranked in the less productive group, although these values reached more than threefold in site C-9 than in site C-36. In site C-36, it yielded a high proportion of lower size graded tubers (44%); a low proportion of tubers with size grade of 45-65 mm diameter (4%), the lightest tubers (31 g/tuber), a low proportion of distorted tubers (1.7%) and spraing symptoms (5%). *Cunera* appears to be the tested cultivars less vulnerable to produce either tuber flesh corky symptoms or tuber distortions; however, it appears to be severally affected in yield and yield components, mainly at site C-36.

Cultivar ***Stemster*** (G) was ranked in the most productive group, with 20675 kg/ha and marketable valuable (18.2 ton/ha), although there are overwhelming evidences of significant differences of total and marketable valuable yield between sites: C-9 (33.7 ton/ha and 31.2 ton/ha, respectively) and C-36 (7.7 ton/ha and 5.2 ton/ha, respectively). In site C-9, although with high total productivity, *Stemster* had poor tuber quality attributes: 8% were graded in the lower size, a low proportion of tubers within size grade 45-65 mm diameter (54%), 9% were distorted and finally the highest spraing tuber flesh proportion (30%). Conversely, *Stemster* reacted quite differently to TRV-PdC36-2 isolate. This is putatively responsible for both a lower yield and a high proportion of lower size graded tubers (35%), but exhibited lower incidence of tuber flesh corky symptoms (5%) and distortions (1%).

Cultivar ***Picasso*** (H) was a medially productive cultivar, although yield was higher in site C-9 (27.8 ton/ha) than in C-36 (11.2 ton/ha). In site C-36, a high proportion of lower size graded tubers (25%), a low proportion of tubers with size grade 45-65 mm diameter (29%) and light tubers (37 g/tuber) were produced. *Picasso* was not much affected with secondary growths (1/4%), but highly susceptible to develop CRS symptoms in tuber flesh in site C-9 (28%), while in site C-36 no symptoms were recorded. *Picasso* appears to be the cultivar less vulnerable to yield reduction and to produce distorted tubers, but quite vulnerable to a high incidence of tuber flesh corky symptoms, especially in site C-9.

Synthesising, it exists a considerable variation in TRV isolate virulence between sites and in specific interactions between the TRV isolate and the plant cultivar. None of the cultivars revealed good performance in all assessed characters, when subjected to these isolates.

Each potato cultivar has its own particular response pattern to disease infection. The identification of the preponderant factors in the outcome of the disease, besides the influence of its own genotype, have been searched revealing that the differences amongst virus isolates are responsible for differences in susceptibility of cultivars (Maas, 1975; Mojtahedi *et al.,* 2001a,b), or not (Robinson *et al.,* 2004). However, these last authors used two of the three tested isolates, belonging to the same serotype. Moreover, the number of viruliferous trichodorids present in soil and their activity were also identified factors, that depend on the environmental conditions created by edaphoclimatic conditions and cultural practices such as: cropping history of the site (Maas, 1975); irrigation or rainfall during cropping season (Cooper and Harrison, 1973) and soil temperature (van Hoof, 1975). Therefore, it can be speculated that the cultivar behavioural differences between two sites might be due to the virus isolate, although also putatively attributable to environmental differences that influence the numbers and activity of the vector nematodes or the conditions for virus life cycle. Moreover, the virus presence was not confirmed in each tuber, being therefore not possible to attribute the responsibility of all assessed yield modifications to TRV. However, the growers reported quite persistent and aggressive potato symptoms in the previous years. Therefore, the considerations made in this paper are based on the presupposition that the TRV isolate had a preponderant effect on plant phenotype developed by the potato cultivars under analysis.

The evidences collected indicate that the TRV isolates present in the field trials had a variable pathogenic potential over the cultivars under study. Significant effects on yield attributes were registered on eight potato genotypes as putative effects of TRV isolates. These effects included tuber spraing symptomless, as well as the classical spraing symptoms, similarly to referred by Cadman (1959) and Harrison (1968). Generally, the assessed characters of tested cultivars were significantly depreciated in comparison with the typical usual levels of those attributes of these genotypes in the region, which are highly productive cultivars. The TRV effect in some cultivars was a poor quality production due to a significant decrease in tuber size, accompanied by a tuber number increase and also a variable degree of spraing-symptomless but distorted tubers, or spraing symptomatic tubers. Any of the tested cultivars were affected by the above described symptoms, when exposed to both TRV isolates. Similar results had previously been obtained by Dale *et al.* (2000, 2004).

The TRV-PmC9-2 isolate appears more virulent, inducing spraing tubers of all cultivars, than the TRV-PdC36-2 isolate. Similarly, Mojtahedi *et al.* (2001a) reported the response of Russet Burbank and Norkotah cultivars from Oregon and Washington potato fields, to natural infection of three TRV isolates transmitted by *P. allius*, a common vector in the Region. Effects were distinct according to the virulence of the isolate: Pasco (Wa); Umatilla (Or) and Mattawa (Wa) (in decreasing order of virulence of the isolates), regardless the vector density (Mojtahedi *et al.,* 2000).

Conversely, the TRV-PdC36-2 isolate induced higher yield reduction in all cultivars than the isolate TRV-PmC9-2. Xenophontos *et al.* (1998), Crosslin *et al.* (1999) and Dale *et al.* (2000) also revealed that the presence of TRV systemic infection in some cultivars is not related with the typical tuber flesh symptoms. These infected assymptomatic seed can act as a source of TRV to weeds and crops, if the compatible vector species is present in the soil, which has a profound effect on the TRV epidemiology in potato fields.

The cultivars response to different TRV isolates allowed establishing a clue for possible field resistance. There was no evidence of spraing resistance broke down seriously at any site. However, this experiment has to be continued to allow trustable conclusions. The TRV isolates have different biological properties, differing in their virulence on potato cultivars; therefore it is important to determine the cultivar response to different strains of virus, in order to infer about the degree of strain specificity of eventual resistance and thus its durability. In other words, those differences have to be screened when searching for interesting potato genotypes in the field.

This paper reports the first assays on fields naturally TRV infected with commercial potato cultivars in Portugal. As vector species are disseminated in the sampled sites, it was shown that there are real conditions for TRV survival with economic impact in potato crop, in these regions. The present results have potential interest to all potato growing countries.

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